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Assessment of the analgesic effect of Alpha-lipoic acid by three acute pain models

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Abstract

Alpha-lipoic acids are Known as a good analgesic in neuropathic pain, especially in diabetic patients. This research aimed to assess the analgesic activity of ALA by three acute pain models using broiler chicks. We used electrical stimulation, hot water test, and formalin test to elicit acute pain. The up and down method was used to calculate the median effect of the analgesic dose. The ED50 of ALA was 45.18 and 74.56 mg/kg intraperitoneally by electrical stimulation and hot water test, respectively. We demonstrated that the peak of analgesic effect was after one hour by using different doses and different times. ALA at 0, 75, 150, 300 mg/kg intraperitoneally produces a dose-dependent analgesic effect by formalin test. In conclusion: ALA induced analgesic activity, probably by closing voltage-gated calcium and or voltage-gated sodium channels. These outcomes show that therapeutic doses of ALA can affect pain and may mask or reduce nociception induced by acute pain models.

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Introduction

Alpha-lipoic acid (ALA) is a natural particle made-up of a five-membered cyclic disulfide and a hydrocarbon tail that ends in a carboxylic acid group. The alpha-lipoic acid, as a result, is a predominantly hydrophobic particle with an amphipathic charisma because of the carboxylic acid group involved in the ring structure (1). Lipoic acid is found in our food, primarily in animal organs like muscles and liver tissues, and at low or untraceable levels in plant foods like potatoes. On the other hand, Alpha-lipoic acid is desirable when used as a food additive due to its antioxidative activity, which has initially been described. Many articles have indicated its preventive properties in cases such as aging type 2 diabetes and neuropathy (2).

Many studies have confirmed that ALA has analgesic effects for pain. In a study conducted on rats, it was found that alpha-lipoic acid had analgesic effects in the postoperative pain model in two ways. The first method for eliciting pain was the pain model. Surgical Incisional pain model and Mechanical hyperalgesia (3), and to explain the putative mechanism of analgesia, it was revealed that V3.2-type calcium channels play an essential role in mechanical and thermal stimulation in rats and mice (3). A previous study on rats indicates ALA has an inhibitory effect on calcium channels type V3.2 in sensory neurons of the dorsal root ganglia of rats and reported that local injection of ALA reduced the sensation of heat and mechanical stimulation (4). ALA works to relieve visceral pain in rats (5). The analgesic mechanism in this research is due to ALA inhibiting sodium channels, blocking them, and reducing their cellular synthesis, especially sodium channels of type V1.8 located in the sensory nerves of the colon in rats with diabetes mellitus (5). In a study conducted on rats to induce neuropathy by Paclitaxel, ALA relieved the pain by activating the nuclear erythroid2-related factor pathway, one of the most critical proteins with anti-pain and anti-inflammatory activity (6). The Writhing test and Hot plate test were involved in assessing the antinociception effect of ALA for prostaglandins manufacture by inhibiting yeast cyclooxygenase peripherally for the take-up test and centrally for the hot plate test (7). The goal of the present study was to assess the analgesic effects of Alpha-lipoic acid in multi acute pain models in broiler chicks.

Materials and methods

Ross broiler chicks of both sexes were obtained from a local hatchery in Nineveh, Iraq. They were housed in batches of 20-25 chicks at a temperature of 25-30 ºC with 24 h lighting and wood shavings as floor litter. The supply of water and feed were *ad libitum*. Experiments were conducted when the ages of the chicks were between 7-9 days. This is a suitable experimental animal (chick) model used previously in pharmacological and toxicological studies (8-11). The Alpha lipoic acid (Lipoic-Forte by AMS®), formalin, and their suppliers were as follows. Each Alpha-lipoic acid was prepared freshly as a solution in propylene glycol and dosed by a needle intraperitoneally in a volume of 5 ml/kg body weight (10). The choice of the ALA dose was based on our initial trials in chicks without producing overt signs of half-hour after the administration of propylene glycol (control) or ALA.

Ethical approval

We obtained the official approval for the study protocol from the Committee of Postgraduate Studies at the College of Medicine, University of Mosul, Iraq, according to institutional regulations on animal handling and used in research.

Experimental animals models of pain

Determination of the median effective dose (ED_{50}) corresponds to a degree of the influence of a drug, being the dose of medicine necessary to yield 50% of that drug's maximal effect of ALA for the induction of analgesia chicks electrical and thermal stimulation. Chicks at 7-9 days old were used. This method is summarized by injecting a chick with a dose of Alpha-lipoic acid and then examining the analgesic effect 15 minutes after the injection. If analgesia appears, the chick is given an X mark, and if it does not happen, the chick is given the mark O, and by repeating this method up and down the dose by a fixed amount (20mg/kg) after the effect change was occurring enabling us to calculate the median effective dose (ED_{50}) of Alpha-lipoic acid based on the table mentioned (12) and Using the following equation: $ED_{50} = Xf + Kd$. Whereas; Xf = the last dose used in the experiment, K = a tabular value extracted from the table mentioned by Dixon (12), d = the constant increase or decrease in the administered dose.

Electrical stimulator test

The up-and-down method was utilized to discover the ED_{50} of Alpha-lipoic acid to generate analgesia in chicks individually. Analgesia was measured by the rise in the pain threshold with an electrical stimulation device (12). The device's electrodes were softly placed at the skin of the chest area, moistened with gel, below the wing. The sign of pain after electric stimulation was detected as struggling and wing-flapping (13). We gradually increased the voltages (starting from 1 volt) to the point where the pain sign appears, and the pain stimulating voltages were recorded for all chicks.

Hot water test

This test is based on the principle of thermal stimulation, where we used a water bath device, and its temperature was set at 55-56 Cº by the internal thermostat of the device. The chick was grabbed gently in one hand, his left foot was placed under the fingers, and the right foot was left free to move and dipped to before the tarsal joint. A stopwatch measured the time spent per second. If the chick does not respond to the thermal stimulus within 20 seconds, it is immediately raised, and its right foot is placed in water at room temperature for 15 seconds to quickly reduce the leg temperature and was then seen for possible heat-induced burns (14).

The analgesic effect of Alpha-lipoic acid in chicks by HWT

Twenty-four broiler chicks were randomly distributed for four groups of 6 chicks each. Chicks in groups of four were treated with Alpha-lipoic acid at 0, 75, 150, and 300 mg/kg. They were immersed individually in a water bath maintained at a temperature of 55 ± 0.5 ºC. The latency to withdrawal the foot from hot water was noted as response time. The response time was distinguished at 0, 15, 30, 60and 120 minutes. The cut-off time was set at 20 sec to escape hurt to the skin.

The analgesic effects of Alpha-lipoic acid in chicks exposed to the formalin test

30chicks were distributed into five groups of 6 birds each. Acute pain reactions were provoked in the chicks by injecting 0.05 ml of 0.1% aqueous solution of formalin into the isolated area of the left foot (15,16). The chicks were treated with either propylene (negative control) or meloxicam 5mg/kg IM (positive control), or Alpha-lipoic acid at 75, 150, and 300 mg/kg 30 minutes previously the formalin injection. Later the formalin injection, we documented within 3 minutes the latency to lift the right foot (using a stopwatch) and the repetition of lifting the left foot in reaction to formalin irritation.

Statistical analysis

The statistics software package SPSS (IBM) for statistical analysis of the data was used. Parametric data were analyzed by one-way analysis of variance followed by the least significant difference test. Data were expressed as mean + SE (standard error), $P<0.05$ was considered statistically significant.

Results

The median effective analgesic (ED_{50}) values of Alpha-lipoic acid in the chicks by electrical and thermal stimulation were 45.18 and 74.56 mg/kg IP, respectively (Tables 1 and 2).

The analgesic effect of Alpha-lipoic acid in chicks by thermal stimulation

Alpha-lipoic acid at 150 and 300 mg/kg created an analgesic response half an hour after injection compared to the control group, and the peak time for analgesia was after 60minutes of ALA injection (Table 3, Figure 1).

Table 1: Median effective dose (ED_{50}) of ALA injected IP for generation of analgesia in chicks by using electrical stimulation

Variables	Results
ED_{50}	45.18 mg/kg
Average of the doses	100-60=40 mg/kg
first dose	100 mg/kg
final dose	60 mg/kg
+ or - in the dose	20 mg/kg
No.of chicks involved	(XXXOXOX) 7
Sings of nociception	Calling and wing flapping

X: Positive reaction of analgesia, O: Negative reaction of analgesia, The ED_{50} were calculated by the up-and-down method.

Table 2: Median effective dose (ED_{50}) of ALA injected IP for generation of analgesia in chicks by using thermal stimulation

Variables	Results
ED_{50}	77.56 mg/kg
Average of the doses	100-60=40 mg/kg
first dose	100 mg/kg
final dose	60 mg/kg
+ or - in the dose	20 mg/kg
No. of chicks involved	(XOXXO) 5
^{o}C water bath	55-56 ^{0}C
Sings of nociception	Foot withdrawal

We calculated the time needed to foot withdrawal from a water bath before and after15 min of ALA injection. X: Positive reaction of analgesia, O: Negative reaction of analgesia, The ED_{50} were calculated by the up-and-down method.

The analgesic effect of Alpha-lipoic acid in chicks exposed to formalin test

ALA at 75, 150, and 300 mg/kg, dose-dependently induced analgesia against acute pain produced by formalin injected into the plantar area of the foot of the chick. The significantly increased latency revealed this to lift the left foot and the significantly decreasing rate of foot lifting compared with the negative control group and positive control group (Table 4)

Table 3: Effect of ALA (75, 150, and300 mg/kg) on thermal stimulation in chicks

	The time (s) needed to remove foot from water bath after ALA injection (min)				
	0	15	30	60	120
Control group	1.37±0.09	1.73±0.19	1.63±0.19	2.63±0.50	2.19±0.30
75 ALA mg/kg	1.80±0.14	2.30±0.19	6.89±1.33*	11.90±2.68*	4.45±0.90*
150 ALA mg/kg	1.53±0.24	3.41±1.42	9.90±2.50*	10.55±1.68*	3.13±0.62
300 ALA mg/kg	1.55±0.22	3.26±0.60	10.23±2.90*	10.12±2.60 *	2.46±0.36

Values are mean ± SE of 6 chicks/group. *Significantly different from the respective control group, P<0.05.

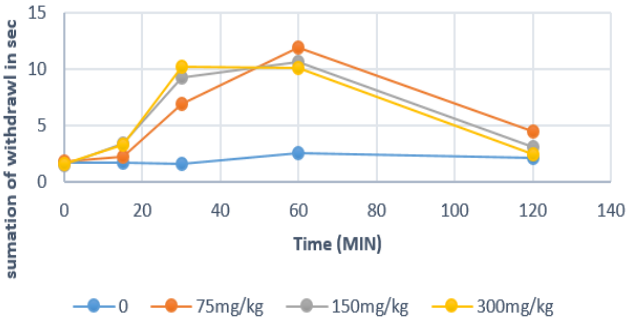

Figure 1: Analgesic effect of ALA at 0 (propylene glycol), 75, 150, and 300 mg/kg IP. next 15, 30, 60, and 120 minutes of injection. Foot withdrawal was the sign of pain sensation after immersion the foot in the water bath.

Table 4: The analgesic impacts of ALA injected IP in chicks exposed to the formalin test

Groups	Latency to lift left foot (seconds)	Repetition of left foot lifting (numbers)
Control (propylene glycol)	1.0±0.0	42.8±9.9
Meloxicam 5mg/kg IM	1.7±0.2*	17.7±2.3*
75 ALA mg/kg	3.0±0.6*	20.8±5.6*
150 ALA mg/kg	3.5±0.4*	17.2±3.2*
300 ALA mg/kg	8.5±1.5*a	9.7±2.2*a

The values are mean ± SE of 6 chicks/group. * Significantly different from the corresponding control value, P<0.05. a Significantly different from the corresponding value of the group treated with meloxicam at 5 mg/kg, P<0.05.

Discussion

Alpha-lipoic acid was used as a feed supplement in poultry and other farm animals. It is characterized by super antioxidant effects and helps as a free radical scavenger, thus increasing the animals' protection against diseases and imbalances that occur in the body due to climatic conditions and pollution on the planet (17). Alpha-lipoic acid has not been studied extensively in the Birds model, and most of the studies were focused on exploring its effectiveness as feed additives. In other laboratory models such as rabbits (18), rats (3), and mice (19), some pharmacological properties have been studied, such as analgesic activity in rats and mice and anti-inflammatory properties. The analgesic properties of the Alpha-lipoic acid and the doses that lead to pain relief in chicks have not been studied previously.

Our current study determined the median effective analgesic doses of ALA using two acute pain models: electrical stimulation and thermal stimulation. Activation of the Mechano-thermal pain receptor results by raising the skin temperature, and the stimulation is concentrated in the form of a prick. As for thermal stimulation by using the water bath test, it is an optimal model for stimulating thermal pain receptors. One of the limitations of our research is that we do not use mechanical stimulation because the device for stimulating mechanical pain receptors is not available (Foot pressure test) (20), and to confirm the analgesic effect of pain, a formalin test was used, which is one of the necessary acute pain tests that can be used in mice, rats, and chicks. The peak analgesic effect of lipoic acid was one hour after injection when using the water bath test. This result was harmonized with the study conducted on rats, which was interpreted by its long duration of action (3). There are several theories about the analgesic mechanism of alpha-lipoic acid. In a study conducted on rats, alpha-lipoic acid at a dose of 60 and 120 mg/kg body weight intraperitoneally analgesic effects in the postoperative pain model. Surgical methods in two ways. The first method for pain induction was the incisional pain model and mechanical hyperalgesia (3). To explain the supposed mechanism of analgesia, it was revealed that calcium channels, V3.2 play a role Important in mechanical and thermal stimulation in rats and mice (21,22). A previous study conducted on rats indicates ALA has an inhibitory effect on calcium channels V3.2 type in Sensory neurons of the dorsal root ganglia of rats, as well as local injection of ALA, reducing the sensation of heat and mechanical stimulation (4). ALA works to relieve visceral pain in rats (5).

The analgesic mechanism in this research is due to ALA's inhibition of sodium channels, blocking them, and reducing their cellular synthesis, especially sodium channels of type V1.8 located in the sensory nerves of the colon. In rats with diabetes mellitus. In a study conducted on rats to induce neuropathy by Paclitaxel, ALA relieved the pain by activating the nuclear erythroid2-related factor pathway, one of the essential proteins with anti-pain and anti-inflammatory activity (6). The Writhing test and Hot plate test were used to assess the analgesic effect of ALA when administered to rats at doses of 10, 25, and 50 mg/kg body weight by oral administration. The manufacture of prostaglandins by inhibiting yeast cyclooxygenase peripherally for the take-up test and centrally for the hot plate test (7).

Conclusions

Alpha-lipoic acid-induced analgesic responses during the electrical stimulator, hot water test, and the formalin test, the analgesic effect may be attributed to the closure of the calcium channel and or closure of sodium channels. The analgesic effects of ALA observed in the current study may be has a clinical benefit for the management of pain in the other animal's model.

Conflict of interest

The authors declare that they have no potential conflicts of interest.

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تقييم التأثير المسكن لـحمض الالفا ليبويك بواسطة ثلاثة نماذج للألم الحاد

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الخلاصة

يعد حمض الألفا ليبيوك من اهم المسكنات المستخدمة لعلاج الاعتلال العصبي الناتج عن مرض السكري. هدفت دراستنا إلى تقييم الفعل المسكن للألم لحمض الألفا ليبويك بواسطة ثلاث نماذج للألم الحاد باستخدام أفراخ الدجاج اللاحمة. تم استخدام جهاز المحفز الكهربائي وجهاز الحمام المائي واختبار الفورمالين لإحداث الألم الحاد بالإضافة إلى استخدام طريقة الصعود والنزول لإيجاد الجرع المؤثرة الوسطية لتسكين الألم لحمض الألفا ليبويك. كانت الجرع المسكنة لحمض الألفا ليبويك ٤٥,١٨ و ٧٤,٥٦ ملغم /كغم من وزن الجسم عن طريق الحقن في الخلب باستخدام التحفيز الكهربائي و التحفيز الحراري على التوالي و كانت ذروة التأثير المسكن لحمض الألفا ليبويك عن طريق الحقن في الخلب بعد ساعة من حقنه، وكان حمض الألفا ليبويك بالجرع ٧٥ و ١٥٠ و ٣٠٠ ملغم/كغم من وزن الجسم في الخلب ذو تأثير مسكن و معتمد على الجرعة في اختبار الفورمالين. نستنتج أن حمض الألفا ليبويك له تأثير مسكن وألية التسكين قد تكون من خلال تثبيط قنوات الكالسيوم ذات الجهد الكهربائي و / أو تثبيط قنوات الصوديوم ذات الجهد الكهربائي، وتشير نتائجنا إلى أن الجرعات السريرية من حمض الألفا ليبويك يمكنها إخفاء الألآم الناجم عن نماذج الألم الحاد.